A Phase I, Open-Label Clinical Trial of the Safety and Tolerability of Single Escalating Doses of Autologous CD4 T Cells Transduced with VRX496 in HIV-Positive Subjects

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VIRxSYS - The Company

- > Established 1998
- > 40 Employees
- > Gaithersburg, Maryland
- Company's mission is to develop gene therapies for serious disease states
- Present focus is the development of a gene therapy for the treatment of individuals with AIDS





Target disease: HIV-AIDS

- > 40 million infected with HIV worldwide
- ➤ 1 million infected with HIV in United States
- ➤ Highly Active Antiretroviral Therapy (HAART) suppresses HIV infection but is not a cure
- ➤ Current HAART is toxic → cumulative failure to therapy
- > Resistance to HAART is on the increase
- VIRxSYS' approach is to use HIV vectors with anti-HIV payloads to interfere with wt-HIV replication



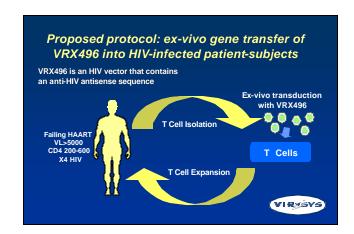
HIV infection is the appropriate disease target to first test HIV vectors

- Testing of HIV vectors in non-HIV infected individuals would result in their seroconversion
- ➤ If non-HIV infected individuals became infected with wt-HIV → ambiguity as to the source of infection
- > HIV-infected patient-subjects are laden with wt-HIV
- Selected patient-subject population has no good treatment options left – failing HAART therapy,
 VL>5000 and show X4 strain of HIV

HIV vectors are the appropriate lentiviral vectors for clinical testing

- The biology and pathogenesis of HIV in humans is well understood, in contrast to other lentiviruses
- HIV infected population with no good treatment options can be identified
- The introduction of non-HIV lentiviral vectors into humans, particularly those infected or at risk of being infected with HIV, would result in unpredictable consequences





Schematic representation of HIV-based vectors PNL4-3 (9709bp) VRX496 (4344bp) VRX494 (4877bp) VRX494 (4877bp)

Safety features of VRX496 for gene transfer into HIV-infected patient-subjects

Safest approach for gene transfer in HIV-infected individuals

- ➤ Vector entirely derived from wt-HIV NL4-3
- > No sequences other than HIV are introduced into patients
- ➤ Patient-subjects are laden with wt-HIV → vector backbone is constructed from conserved regions of the virus
- > NL4-3 derived from strains common to North America

Evidence for safety from SIV infection in non-human primates

- Attenuated SIVs that are deleted in accessory genes do not cause disease in non-human primates
- No HIV accessory genes in either VRX496 or VIRPAC
- Although attenuated SIVs containing IL-2 can exacerbate disease, no such reports for attenuated SIVs without IL-2
- > VRX496 does not encode for a gene (e.g. IL-2)

Additional safety features of VRX496 for gene transfer into HIV-infected patient-subjects

- Targeted expression of anti-HIV antisense payload antisense payload is Tat and Rev dependent and thus expressed only after wt-HIV infects vector containing cells
- Antisense payload appears to decrease VRX496 mobilization to cells - expression of anti-envelope antisense results in decreased mobilization of packaged VRX496 genomes
- ➤ VRX496 contains a stop codon in gag recombination with helper or wt-HIV would result in a non-functional gag-pol open reading frame



A stop codon in VRX496 prevents translation of gag-pol upon recombination degenerated gag (no psi) gag pol rev tat gag stop psi cppt AS RRE tag

Possible recombination events between VRX496 and wt-HIV GAG STOP TRUNCATED ENV, NO TAT TRUNCATED GAG & POL Result: non-infectious recombinant or wt-HIV

Safety of VRX496 for use in HIV infected patient-subjects

- The backbone of VRX496 contains regions of HIV that are highly conserved (LTR, psi/gag, cPPT, rre)
- Only the antisense payload contains sequences that are not highly conserved (X4 tropic gp120 sequences)
- Restricted the patient-subject population to those that show X4 strain of HIV
- Not claiming that mobilization of VRX496 is required for anti-HIV efficacy, but may provide some additive effect
- VRX496 is a maximally gutted vector with poor mobilization characteristics while maintaining high transduction and anti-HIV efficiencies

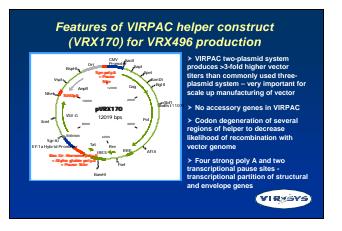


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Self inactivating (SIN) HIV vectors are not optimal for use in AIDS gene therapy

- > Loss of targeted Tat & Rev dependent expression of the antisense payload sequence
- > Constitutive expression of anti-HIV antisense payload may be detrimental to the host cell
- Replacing or modifying the HIV sequences 5' and 3' to the antisense may affect trafficking of the RNA to site of genomic wt-HIV RNA accumulation and decrease anti-HIV efficacy
- > Competition for packaging between vector and wt-HIV genomes is important for anti-HIV efficacy. A SIN HIV vector, by definition, would not compete with wt-HIV for packaging
- ➤ Cannot guarantee that SIN vectors will not mobilize

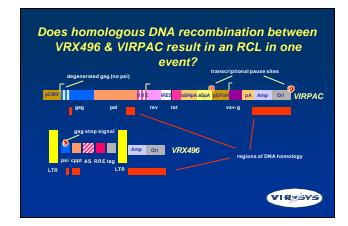


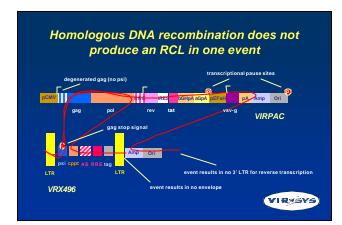


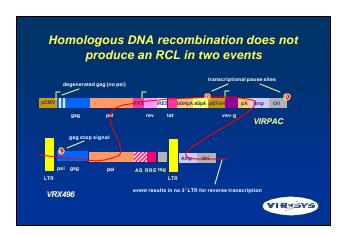
Safety of VIRPAC plasmid system for HIV vector production

- > Theoretically, there is always a possibility for VSV-G and HIV to recombine regardless of the vector production system
- ➤ Minimize potential for vector and helper recombination by intelligent construct design - multiple events needed to obtain a putative RCL
- ➤ Minimize potential for homologous DNA recombination and reverse transcriptase-mediated RNA recombination
- > Strategies other than "splitting plasmids" are available to minimize potential for recombination

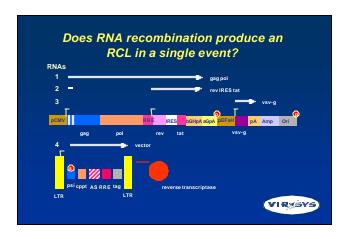


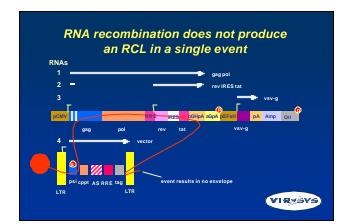


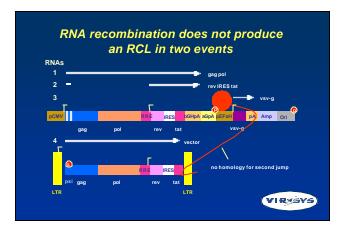


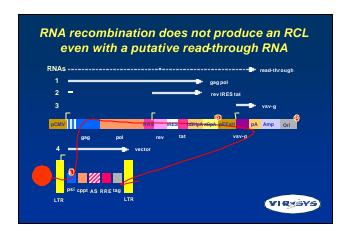


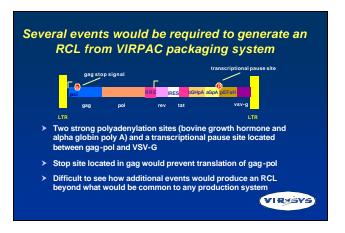
Several events would be required to generate an RCL from VIRPAC packaging system Transcriptional pause sites gag stop signal pair rev tat vsv-g Two strong polyadenylation (bovine growth hormone & alpha globin) & a transcriptional pause site located between gag-pol and VSV-G Stop site located in gag would prevent translation of gag-pol Difficult to see how additional events would produce an RCL beyond what would be common to any production system No 3'LTR







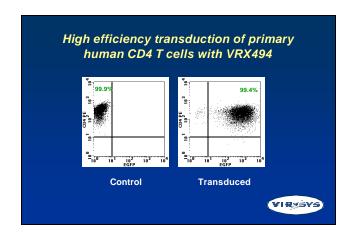


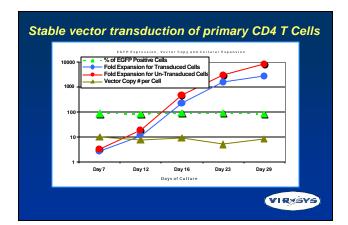


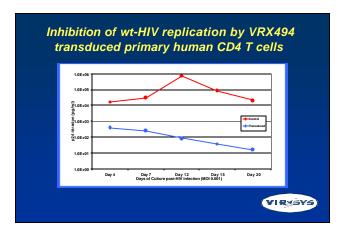
Comparison between VIRPAC and stable producer cell lines

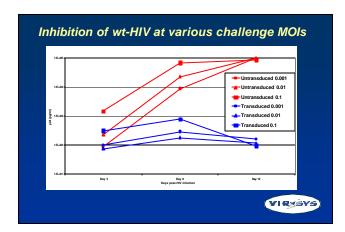
- No producer cell line has been described that can produce a vector titer of at least 10⁷ TU/ml of cGMP grade HIV vector needed for the proposed phase I clinical trials
- VIRPAC offers advantages
 - > Can produce sufficient amounts of clinical grade vector from a scale-up manufacturing process
 - Multiple recombination events would be required to generate a putative RCL
- VIRPAC contains safety features comparable to other transient production systems and producer cell lines

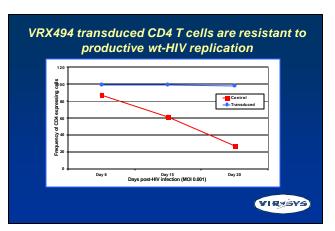


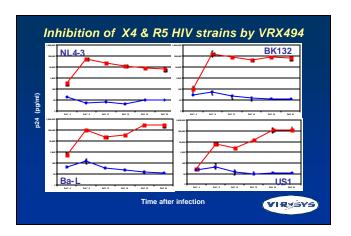


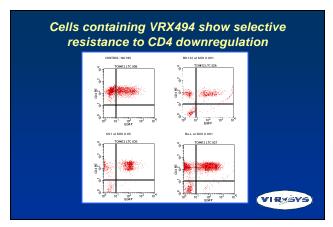




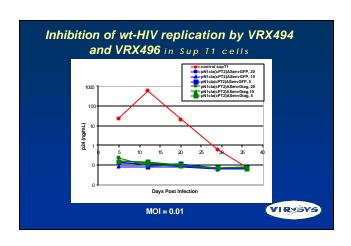


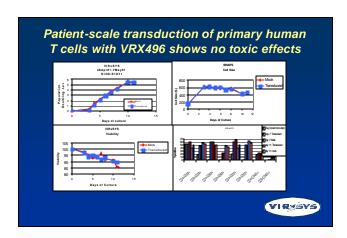


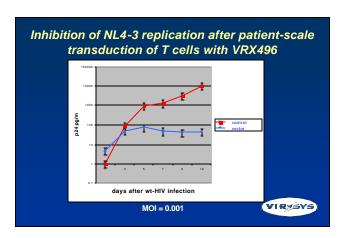


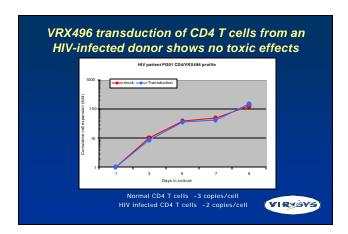


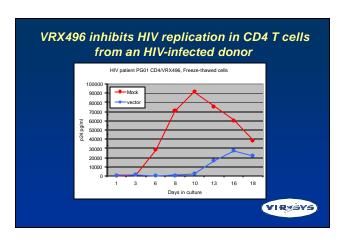
Summary of the in vitro transduction and wt-HIV challenge data High transduction efficiencies can be attained in primary human CD4 T cells Over 99% of wt-HIV can be inhibited upon challenge of vector containing primary T cells with a variety of MOIs Both X4 and R5 strains of HIV can be effectively inhibited by the 937nt antisense payload targeted to the HIV envelope Cells transduced with vector show selective resistance to CD4 downregulation and hence, to productive wt-HIV replication

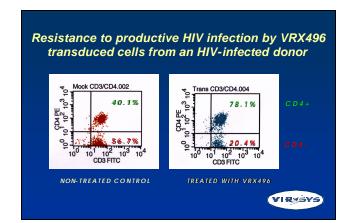


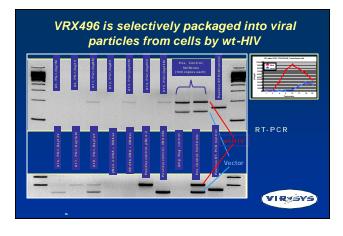


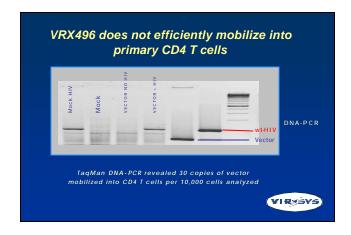


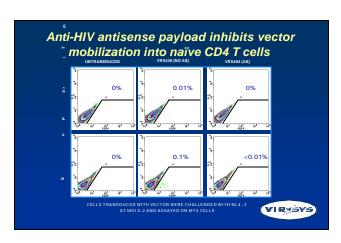


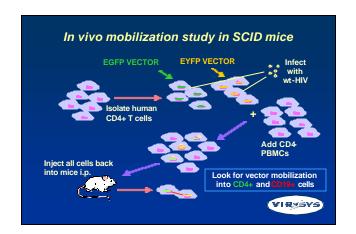


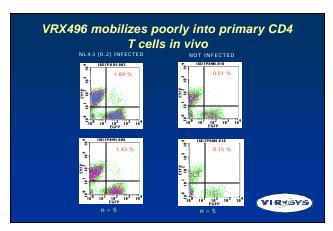


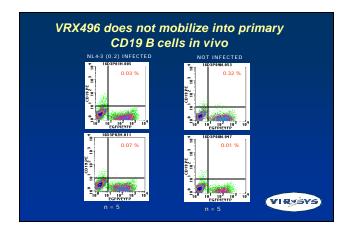










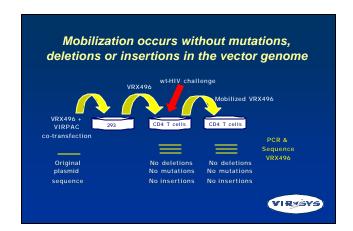


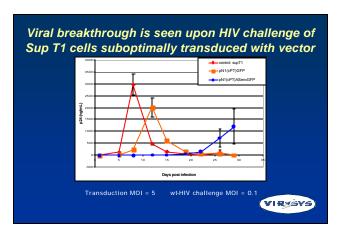
Summary of the in vitro and in vivo mobilization data

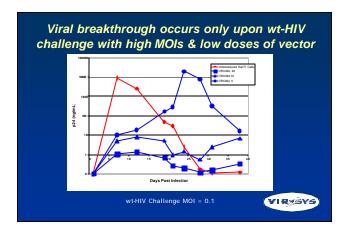
Mobilization is only a safety concern when vector spreads beyond the intended target tissue, CD4 T cells

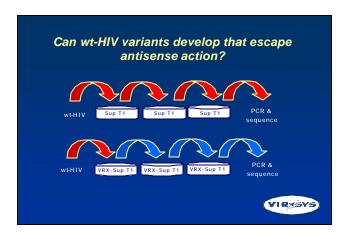
In vitro and in vivo data show that VRX496 mobilizes poorly between primary CD4 T cells

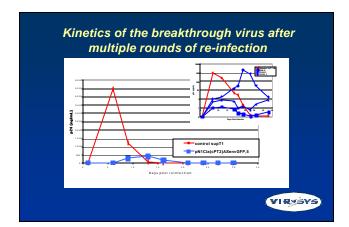
In vivo data shows that VRX496 does not mobilize beyond CD4 T cells, the intended target tissue → no mobilization was seen into CD19 B cells (CD4 negative)

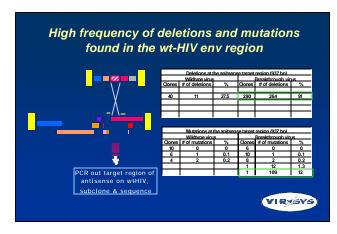


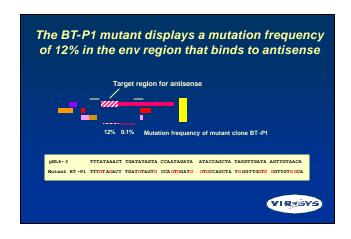


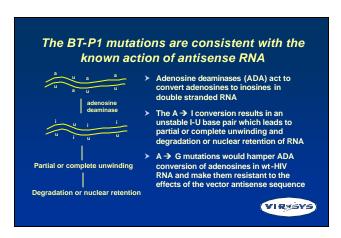










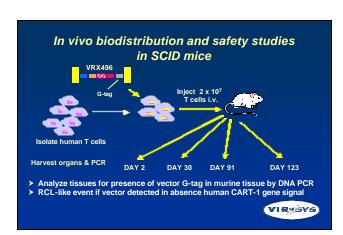


The BT-P1 mutations significantly affect virus replication when cloned back into wt-HIV 5000000 4000000 TCID₅₀ 1.3 x 10⁶ 3000000 wtHIV Virus Titer 2000000 □mutant undetected 1000000 1st ROUND 2nd ROUND The BT-P1 mutation severely restricts viral replication The BT-P1 mutant likely persists by pseudotyping with wt-HIV VIRESYS

Summary of the viral breakthrough data

- Initial breakthrough virus is due to high MOI of wt-HIV overcoming suboptimal transduction levels in Sup T1 cells
 → no breakthrough when sufficient doses of vector are used
- A variant HIV can be selected that shows increased resistance to vector inhibition → however, the consequence of this resistance is a decreased fitness for replication
- Very high deletion and mutation frequencies are seen at the target env region on wt-HIV strongly indicating that the vector is acting upon wt-HIV by the antisense base-pairing mechanism

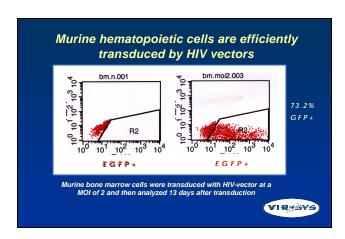




Advantages of using SCID mice for biodistribution and toxicity studies

- Introduction of human cells containing HIV vector into an animal model -> not possible for any other animal model system
- Injected human cells survive for long periods of time in the animal, permitting amplification of an adverse event in the human cell population that is resident in the animal
- Human cells eventually die in the animal permitting visualization of adverse events in whole tissues by PCR and other methods
- HIV vectors can transduce murine cells efficiently which is likely sufficient for detection of an overt adverse event in mouse tissue despite lack of productive HIV replication

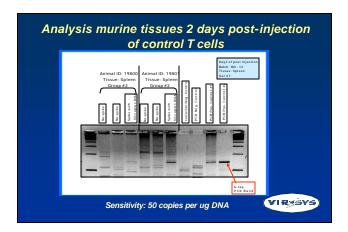


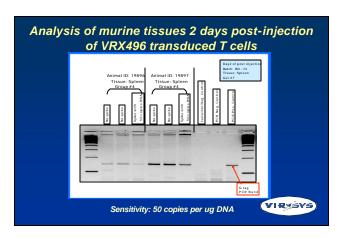


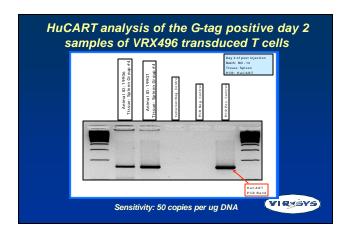
SCID vector biodistribution study design

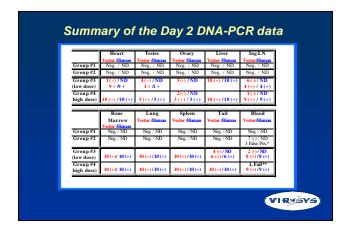
# Of mice	Animal group#	Day2	Day15	Day30	Day90	Day123
Infusion Media control	1	5	5	5	5	5
Mock transduced control T cells 20 x 10°/mouse	2	5	5	5	5	5
Vector transduced T cells (low dose) 3 x 10 ⁵ /mouse	3	5	5	5	5	5
Vector transduced T cells (high dose) 20 x 10 ^s /mouse	4	5	5	5	5	5
Total for male mice		20	20	20	20	20
Total for female mice		20	20	20	20	20
Total number of mice		40	40	40	40	40

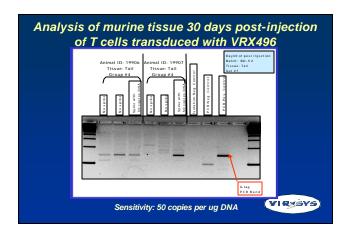


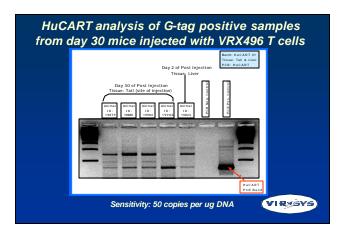


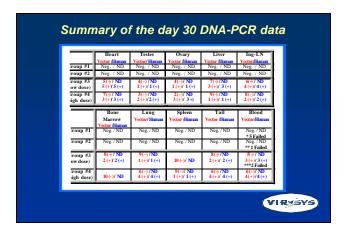


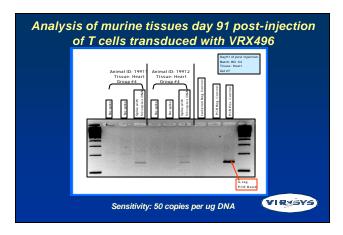


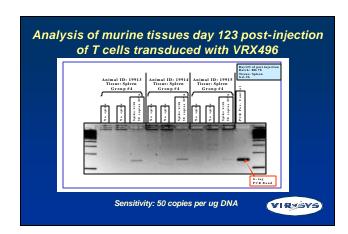










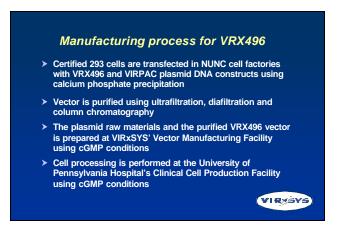


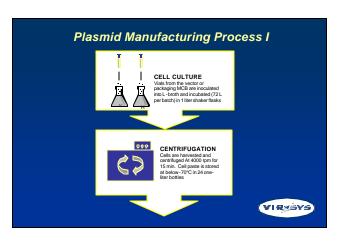
Summary of the day 123 DNA-PCR data Testes Liver Group #1 Neg. / ND Neg. / ND Neg. / ND Group #4 5(-)/ND ND 5(-)/ND ND Lung Tail Blood Neg. / ND Neg. / ND Group #1 Neg. / ND *8 failed (low dose) ND 10 (-) /ND Group #4 (high dose) 10 (-)/ND 10 (-)/ND VIRSSYS

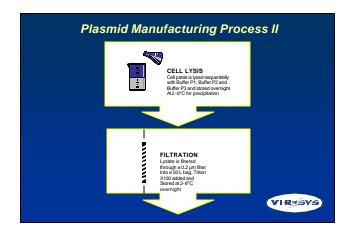
Summary of the animal biodistribution and toxicity data

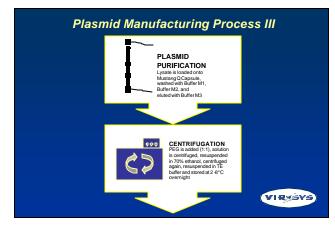
- Infused human T cells containing vector could survive for up to 91 days in SCID mice
- ➤ In all tissues studied a G-Tag vector signal was associated with a Hu-Cart-1 signal for human DNA → G-Tag signal is due to vector containing T cells
- A total of 6 animals from the study displayed clinical manifestations that were not treatment related
- > No adverse RCL-like events were observed

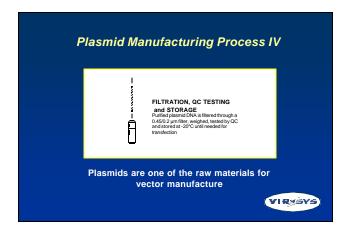


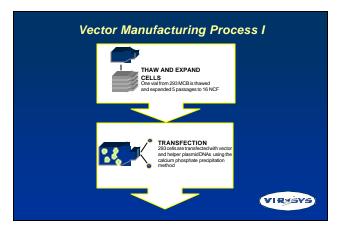


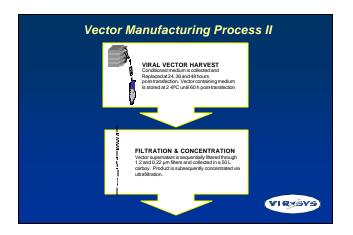


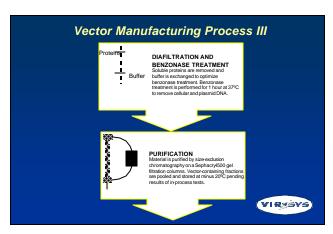




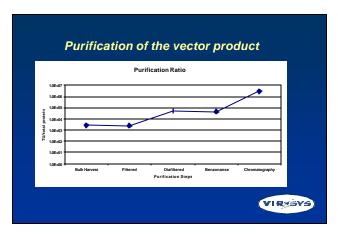


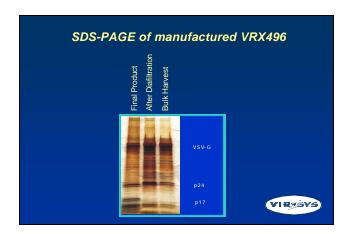


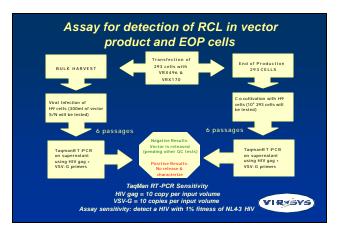


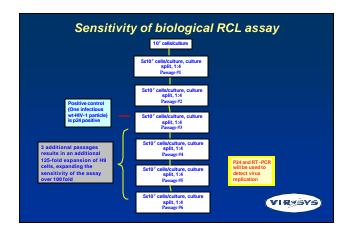








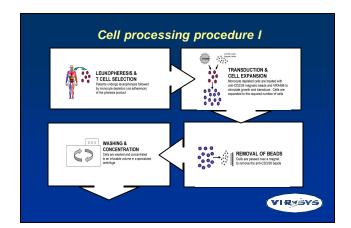


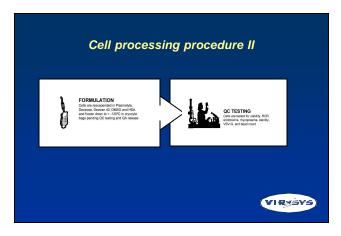


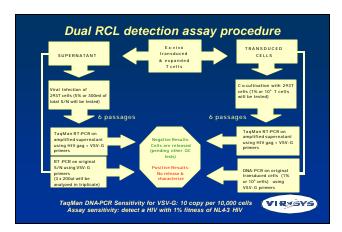
Should an in vitro assay for detection of functional LTR-gag-pol-LTR be used as lot release assay?

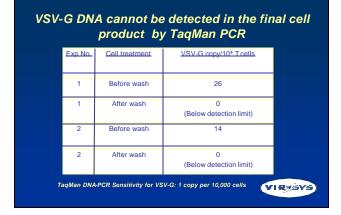
- > The assay may have utility for assay of HIV vectors for non-HIV disease applications
- > For HIV disease, the final cell product already contains similar events in abundance
- ➤ We have found that vectors similar to proposed detection event can inhibit wt-HIV replication → so presence of the event in the final cell product may not be a detrimental one in AIDS gene transfer

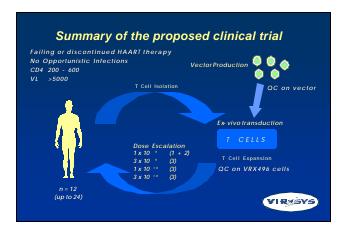


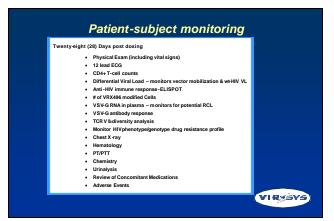












Patient-subject monitoring

- > Patients will be monitored short term at 24, 48 & 72 hours, 7, 14 & 28 days and long term at 3, 6 & 12 months and yearly for life
- > Dose escalation would proceed after 28 days because most of the short lived activated T cells would have died within a few weeks, the cells most capable to support wt-HIV replication or the replication of a putative HIV recombinant
- ➤ Long lived T cells are normally quiescent and not capable to support productive HIV replication while in their quiescent state

 → would support productive HIV replication upon their sporadic activation with antigen → long term follow-up at 3, 6 & 12+ Mo.
- Since activated T cells are most abundant immediately after infusion, the greatest risk for an adverse event is short term



Summary I

- ➤ HIV vectors can transduce at >90%, inhibit wtHIV replication by over 99% and provide CD4 T cells with selective resistance to production wt-HIV infection
- > VRX496 is a fully gutted vector with no novel sequences in the vector → even the antisense payload is derived from wt-HIV
- ➤ VRX496 does not mobilize beyond the target tissue → in vitro and in vivo studies show poor mobilization occurs only between CD4 T cells
- > Vector production methods use vector packaging systems that are comparable to those used in other gene transfer studies
- Animal biodistribution and toxicity studies show the vector to be safe

Summary II

- > Final cell product release testing criteria are highly stringent

 - no detection of VSV-G DNA in final cell product
 no detection of VSV-G RNA in final cell supernatant
 - > no detection of an RCL after biological amplification in a highly permissive human cell line and detection by TaqMan RT-PCR
- > Treating HIV infected individuals with advance disease that have no good treatment options left affords the lowest risk for testing HIV vectors and the highest chance for benefit
- Drug therapy failure due to toxicity is common and viral resistance to these drugs is increasing
- Urgent need to develop new strategies to treat individuals with AIDS → biological control of HIV with an HIV vector may offer new treatment opportunities for individuals with AIDS



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